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A Comprehensive Review on the Bioactive Properties of *Antigonon Leptopus*

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ABSTRACT

Antigonon leptopus Hook. & Arn, commonly called chain of love, is a fast-growing, leafy vine bearing pink or white, heart-shaped flowers. It is one of the most invasive vine species on Guam. Although the vine is regarded as a weed in Guam, it is used in various folk medical techniques around the world, including treating wounds and relieving colds. The underutilization of *Antignon leptopus* as a resource in Guam has prompted the investigation of potential pharmacological characteristics. Numerous phytochemicals found in *Antignon leptopus*, such as alkaloids, terpenoids, flavonoids, phenols, tannins, saponins, glycosides, and amino acids, have been shown to have a variety of bioactive qualities, such as antioxidant, antimicrobial, antidiabetic, anti-inflammatory, and anticancer effects. In numerous areas worldwide, the seeds, tubers, and flowers of the plant are employed as sustenance sources. Tea made from aerial parts is beneficial for pain alleviation and treating common ailments. The leaves are used to treat diabetes, low blood pressure, and urinary difficulties. They are also a heart tonic. It has anticancer and xanthine oxidase inhibiting effects. It originated in Mexico and is now found globally, including in India. This review aims to provide current information on the pharmacological activity, phytochemistry, and therapeutic uses of *Antigonon Leptopus* Hook & Arn. The herb is traditionally used to treat a variety of ailments, including stomach aches, flu, diabetes, pain, coughing, and dermatological disorders. Several components of the plant are being investigated for their pharmacological characteristics. It is used in ethnomedicine and that the plant may be used to create pharmaceuticals to cure illnesses.

KEYWORDS: *Antigonon leptopus, Phytochemicals, Underutilization, Weed, folk medical, leafy vine, Extractions, Invasion, enzyme inhibition.*

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INTRODUCTION

Antigonon leptopus, also called Confederate vine or Mexican creeper, is a fascinating climber originating from Mexico and Central America. This ornamental plant, from the Polygonaceae family, has gained much interest due to its brilliant flowers, which change throughout the season, and due to the fact that this plant shows adaptable growth patterns, being considered an ideal species for gardens, landscapes, and even a houseplant (Elhaj et al., 2015*).Antigonon leptopus* looks very distinctive and captivating, with flashy pink or white flowers like clusters of hearts, for which this plant got its cute name, "coral vine." Jacob et al., 2001. Apart from the aesthetics, this adaptable plant has been the focus of considerable scientific research; accordingly, scientists investigate possible therapeutic and ecological applications of this plant. Though *Antigonon leptopus* is an aggressive growth, especially in disturbed areas, its ability to thrive in a wide range of temperatures and soil conditions has made it a staple addition in many gardens and landscapes today. This interest will be reviewed with regard to history, botanical properties, and multiple uses in the subsequent sections of this article.(Balasubramani et al., 2015). Although

Antigonon leptopus can be an aggressive growth, especially in disturbed areas, its capacity to flourish in a wide range of temperatures and soil conditions has made it an important addition to many gardens and landscapes (Baral, 2011). In this detailed study article, we will look at the history, botanical properties, and various applications of this interest.

Table 1. Taxonomic Classification for *A.leptopus*

Rank	Scientific Name and Common Name
Kingdom	Plantae-Plants
Division	Magnoliophyta-Flowering plants
Class	Magnoliopsida-Dicotyledons
Subclass	Caryophyllidae
Order	Polygonales
Family	Polygonaceae Juss.-Buckwheat family
Genus	*Antigonon* Endl.-Antigonon
Species	*Antigonon leptopus* Hook.& Arn.-Coral vine

BOTANICAL DESCRIPTION

The Polygonaceae family consists of about 1200 members from 50 genera. *A. leptopus* is one among several species in this family that are used as salads, culinary items, and ornamental plants. *A. leptopus* has several scientific names, such as Corculum leptopum (Hook. & Arn.) Stuntz and Corculum leptopus (Hook. & Arn.) Stuntz, Antigonon cinerascens M. Martens & Galeotti, Antigonon cordatum M. Martens & Galeotti, and Antigonon platypus Hook. & Arn. Among them, the most frequently occurring ones is *Antigonon leptopus*, which is commonly called queen's wreath or coral vine (Akmal & Ranjan Srivastava, 2019). It is widespread in tropical Asia, Africa, the Caribbean, and the Americas; it is native to Mexico. *Antigonon leptopus* is a forcefully growing perennial woody climber, reaching 5–13 meters length. *Antigonon leptopus* forms a network of rhizomes from which branches grow up to 25 cm every month. The species can also spread by seeds. It gives only a few seeds during sexual reproduction (Ghareeb, 2018*). Antigonon leptopus* can thrive under severe conditions like little light and poor soil. The vine can climb and smother higher vascular plants but can spread laterally over dry land or grasslands through rhizomes, stolons, or tubers. *A. leptopus* is a difficult plant to eradicate because of its huge tubers and rhizomes, hardiness, and several modes of dissemination (Eppinga et al., 2022). They harm natural vegetation, especially on islands. It spreads along the coastline and kills native vegetation. The coral vine dominates the island of St. Eustatius, covering about 20% of the land and interfering with crab migration (Bhatnagar and Pradhan, n.d.; J. M. and D. Burke, 2011). This plant has laterally spreadingstems with switching oval or triangular-ovate leaves. The highest layer of the leaves is shiny and light green, with sunken venation, but the below surface is dull and green, with visible venation. Pink-colored bisexual blooms are commonly found in terminal panicles or axillary racemes. (J. M. Burke & Di Tommaso, 2011) Coralita, normally referred to as coral vine, was introduced to Uttar Pradesh, India from Nepal due to the favorable bioclimatic conditions that permit the growth of similar species (Baral, 2011; Lockwood, 2005; Tiwari, 2005). Balrampur district falls to the east-west and south sides of Nepal. The invasion of coral vine in the district has devastated the native biodiversity. It grows along the road side and depletes other plants hence threatening the local biodiversity and affecting community structures and disrupting ecological services (Akmal & Ranjan Srivastava, 2019). This could be due to temporal dioecism and the promiscuous nature of the flowers, which seem to be adaptive in *A. leptopus* as it enables the species to attain xenogamy while facilitating geitonogamy even though its pollinators are unspecialized. *A. leptopus* is able to thrive as a weed in many kinds of tropical habitats because of its unspecialized blossoms, enabling the plant species to make use of a variety of pollen carriers.

The coral vines are also seen in the coasts of Andhra Pradesh. The tubers are edible, taste nutty, and are consumed in Mexican cuisine (Apaya & Chichioco-Hernandez, 2014). From coral vines, some chemicals can be extracted with pharmacological use in the healthcare system. *A. leptopus* leaves were

traditionally used to cure edema, and from its leaves, a tea can be prepared that is helpful to cure diabetes, and flowers can treat high blood pressure (Avn et al., 2011). People of Thailand commonly eat leaves and pink flowers with cooked vegetables. *Antigonon leptopus* leaves and blossoms may be used to make hot herbal tea (Lim.T., 2013).

Tubers are edible, with a nutty taste, and are used in Mexican cooking (Apaya & Chichioco-Hernandez, 2014). Medicinally, coral vines yield pharmacologically important chemicals. The flowers of *A. leptopus* are used according to Avn et al. (2011) for hypertension treatment, the leaves are traditionally used to alleviate edema, and tea from the leaves can be given for diabetes treatment. The pink flowers are eaten along with leaves as vegetables in Thailand and are usually cooked. *Antigonon leptopus* leaves give hot herbal tea.

ETHNOBOTANICAL USE

The various parts of *A. leptopus* serve various purposes, ranging from food to traditional medicines around the world, with some regions overlapping practices with others.

Mariana Islands

Since *A. leptopus* was introduced to Guam as an ornamental in 1905, its uses are not well recognized there. The plant's distinctive properties as a tea have been studied recently by traditional healers; a herbal tea has been prepared from it in many parts of the world for centuries. Entrepreneurs also tried to find a modern use for the vine: using the roots as a natural dye for cloth, and the flowers in bath bombs and other cosmetics. However, the tuber is said to be used as a starch source in a few tropical countries.

Traditionally, all parts of the vine are used as ingredients in Thai cuisine: blossoms are mixed with omelets and noodles, and leaves are fried after they had been dusted with flour. Due to its antioxidant properties, the aerial parts of the plant-from the vines up to the flowers-are boiled in hot water in order to produce a herbal tea, which is administered to cure colds. In fact, some even claimed that tea produced from the leaves has antidiabetic properties and lowers blood pressure, the authors of a previous study said.

Meanwhile, *A. leptopus* has also been employed in traditional medicinal applications in various parts of India, where it is described to possess several therapeutic and healing properties. The plant's aerial parts are employed to treat common colds, and the leaves, in the form of a paste, treat and relieve blisters, skin irritations, and even illnesses, as stated by the authors of a previous study. The Ifugao tribe in the mountain regions of the Philippines applies *A. leptopus* on open wounds to help with proper healing and reduce inflammation while the Iloilo tribe uses it for gastrointestinal ailments.

Americas

Leptopus tea is also reportedly utilized by Kingston, Jamaica, residents to treat the common cold, as it is in Thailand. However, its preparation methods and plant material used appeared to vary depending on personal preference. Moreover, hot tea has been reportedly employed for generations as a treatment for coughs and throat constriction. According to Vandebroek et al., the vine was used more in cities than in rural areas, possibly due to availability of the plant material. The plant material was traditionally employed as an antihypertensive and in the treatment of diabetes in Trinidad and Tobago. The leaves of the vine have also been reported by authors in ethnopharmacological applications for the treatment of swelling and boil

EXTRACTION PROCEDURE

The leaves of *Antigonon Leptopus* Hook & Arn. were extracted using the protocol described for overnight maceration techniques. Approximately 50 g of spherical material was macerated in 250 ml of ethanol at room temperature for three hours. From time to time, ethanol was shaken at the ratio of 1:10 (1 gram in 10 ml solvent). After a full day at room temperature, the supernatant was decanted. Thereafter, the supernatant was filtered under reduced pressure. The extracts were stored at 4°C until they were needed. MEDICAL ASPECTS Recent developments in complementary and alternative medicine have highlighted their desire to learn more about the medicinal plants of the world.

The phenolic compounds have been increasingly reported to exhibit significant antibacterial, antioxidant, and anti-inflammatory capacities. Tea made from the aerial parts of *Antigonon Leptopus* Hook & Arn is employed to cure a sore throat, cold, and throat constriction. Practitioners of traditional medicine utilize several plants in specific preparations for treating a number of diseases. As many as 80% receive their first line of primary medical attention from medicinal plants, as estimated by a WHO assessment. The traditional medical systems of Ayurveda, Unani, and Siddha are known to rely extensively on plants. Leaves treat menstrual cramps, diabetes, hypertension, and edema, while vines are taken to heal coughs and throat constrictions, as also mentioned in a different study. *Antigonon Leptopus* Hook & Arn has been used in traditional Chinese medicine for treatments such as nephritis, hepatitis, and colitis.

EXTRACTION PROCESS

Following the protocol outlined, leaves of *Antigonon Leptopus* Hook & Arn.[100] were subjected to overnight maceration techniques. Approximately 50 g of spherical material was subjected to 250 ml of ethanol at room temperature for three hours. Intermittently, ethanol was shaken at a ratio of 1:10 (1 gram in 10 ml solvent). Following a full day at room temperature, the supernatant was decanted. The supernatant was filtered under reduced pressure. Extracts were stored at 4°C until needed. MEDICAL ASPECTS Interest in learning more about the world's medicinal plants was brought to the forefront by recent events in complementary and alternative medicine. Phenolic compounds repeatedly showed significant antibacterial, antioxidant, and anti-inflammatory capabilities. *Antigonon Leptopus* Hook & Arn. produces tea from the aerial parts which is used to heal sore throats, colds, and constricted throats. Traditional medicine professionals use several plants in a specific formula to cure a wide range of diseases. Medicinal plants serve as the primary source of medical treatments for nearly 80% of individuals, according to a

WHO assessment. Plants are the main source of most therapeutic agents of traditional systems of medicine like Ayurveda, Unani, and Siddha. Another study of *Antigonon Leptopus* Hook & Arn. mentioned that it is a vine used for cough and throat constrictions while leaves are used to treat menstrual cramps, diabetes, hypertension and edema. It has also been used to treat colitis, hepatitis, and nephritis in Chinese traditional medicine.

EXTRACTION METHODOLOGY FOR BIOACTIVE

Various factors exist, such as the types of solvent used, sample-to-solvent ratios, extraction times, and temperatures that influence the extraction of bioactive chemicals from *A. leptopus*. Water, methanol, ethanol, ethyl acetate, hexane, and chloroform were the commonly used solvents in this research for preparing the resulting extracts by analytical and biological activity testing.

Traditional Extraction Methods

Hot extraction, maceration, Soxhlet extraction, and sequential extractions have been the primary extraction techniques employed in the research of *A. leptopus*.

Hot Extraction

Plants were extracted in research carried out by Balasbrumani, Rudhra, and Venkatesan by combining the dried powder with a solvent and boiling for variable time. For example, in Balasbrumani et al.'s study, 8 g of leaf powder Rudhra and Venkatesan extracted 50 mg of leaf powder with 250 mL of methanol, ethanol, and acetone for 72 hours, while water was used for 30 minutes. Vanisree et al. isolated phenolic components in a manner similar to tea preparation; 50 mL of boiling water was directly poured onto 5 g of aerial parts of the plant and steeped for 6 hours. The resultant tea extract was then dried and tested.

Soxhlet Extraction

Heat extraction was traditionally done by Pradhan and Bhatnagar using a glassware called the Soxhlet extractor, invented by a German named Franz von Soxhlet. Approximately 20 g of *A. leptopus* leaf powder was packed evenly in a thimble and extracted with 250 mL of solvents that were arranged from nonpolar to polar solvents: hexane, chloroform, ethyl acetate, and methanol. Ranjan and Ahmad used Soxhlet extraction in order to study antibacterial and anti-inflammatory activities of *A.leptopus*. They extracted 10 g of powdered materials with methanol at 80 °C for three cycles over 12 hours. Soxhlet extraction involves placing the sample and solvent in a fat extractor and heating the entire device. When the solvent heats up and boils, steam rises through the pipe, condenses into a liquid, and drops into the extractor. Using solvent reflux and siphoning, the soluble matter in the sample is enriched in a flask.(Yasa Gayatri Devi 2025).

Maceration

Maceration is the most popular extraction process for bioactive chemicals from *A. leptopus*. Mamidipalli et al. utilized coarse powder from *A. leptopus* roots and methanol to extract the chemicals at room temperature. On the other hand, Kaisoon et al., employed a maceration technique to study the phenolic constituents and the antioxidant properties of the flowers of *A. leptopus.* To begin with, ethanol was combined with the floral powder and shaken at room temperature (25 °C) for 2 hours before centrifugation for 10 minutes. The supernatant was collected, and extraction of the powder was repeated twice more before evaporating it. Bolla and Bhogavalli say that a 1:10 sample-to-solvent ratio of ethanol and chloroform was involved in extracting the powder of the flower. The powder and the solvent were kept in a stoppered flask at room temperature (25 °C) for 48 hours, with occasional shaking in the first six hours. Similar 1:10 ratios of dried *A. leptopus* leaves and 100% methanol were used by Shantaram and Bollapragada, but they macerated the mixture for only 24 seconds with intermittent stirring. Poosa and Vanapatla extracted their leaf powder by macerating it in methanol for seven days before its concentration, though several researchers who conducted maceration

did so for one to three days. A cold maceration technique was used to study the in vitro cytotoxic activity of *A. leptopus* chloroform extract.

Sequential Extraction

Various researchers have carried out serial extractions with different types of solvents such as methanol, ethyl acetate, deionized water, and so on to analyze various types of phytochemicals and plant extracts. Vanisree et al. carried out successive extractions of the aerial parts of *A. leptopus* using hexane, ethyl acetate, and methanol. Later, the extracts were subjected to silica-gel medium-pressure chromatography (MPLC) to collect various fractions. Apaya et al. extracted the powder using methanol, filtered it, and concentrated it to get a crude methanol extract. Further, the crude extract was partitioned using hexane and deionized water, and the aqueous layer was further extracted using ethyl acetate.

Non-conventional Extraction Methods (Green Technology)

Non-conventional, or "green," extraction methods aim at the effective extraction of bioactive compounds from plants, reducing their adverse impact on the environment, such as the use of harmful solvents and energy consumption. There are not many specific studies related to green extraction techniques applied to *A. leptopus*.

UAE can also be combined with other extraction methods such as S-CO2 allowing for a more effective extracting method. Compared to traditional methods, extraction times can be significantly reduced and extraction takes place at a lower temperature while still maximizing product yields [19,20]. Pereira et al. studied optimized UAE techniques for the extraction of phenolic compounds from Camillia japonica, testing variables such as power, time, and the solvent used. Their optimized method conditions were 62% amplitude, 8 min, and 39% acidified ethanol, which allowed them to obtain a 56% yield [21]. However, optimal extraction conditions are highly dependent on the sample composition.

Supercritical-Carbon-Dioxide (S-CO2) Extraction

S-CO2 extraction is a green technology that can extract bioactive chemicals from plant materials using CO2 as a solvent at above 31 °C and above 7.3 MPa pressure. Recently, SFE has gained much attention due to the number of advantages and sustainable applications over conventional extraction techniques. The properties of the supercritical fluid, low pressure, and moderate temperature required for extraction enable the efficient and selective separation of bioactive chemicals while preserving their functional qualities. Additionally, currently, the use of CO2 by SFE technology in removing hazardous waste materials is being studied to reduce CO2, thus minimizing pollution and its effects on the ecosystem altogether. Currently, there are no specific studies on the application of S-CO2 to *A. leptopus*; however, the method has been successfully applied in the extraction of bioactive chemicals from a range of other plant species. However, the major disadvantage of SFE is its highly expensive equipment, operational costs, and specialized skills required to operate the system. Due to these challenges, the extraction method is not as accessible as other approaches. In addition, some molecules, including non-polar chemicals, cannot be easily extracted by supercritical fluids; hence, SFE is not suitable for all types of extractions.

Microwave-Assisted Extraction (MAE)

Another green technique, which depends on exciting the liquid particles using microwaves is referred to as microwave-assisted extraction (MAE). In this case, the mobility of the liquid enhances the extraction process. MAE techniques have advantages over other conventional techniques for extraction in terms of shorter extraction time, less solvent used, and automation. One of the problems associated with MAE techniques is non-uniform heating, which may affect extractions. This problem can be avoided by using appropriate combinations or evenly dispersing the sample.

Both MAE and UAE can be equally successful for the extraction of high-quality phenolic compounds; however, the method should be chosen according to sample composition and particular extraction goals. Since there is a lack of data and research dealing with the application of MAE approaches to *A. leptopus*, further research may be conducted to identify an optimum method of extraction of *A. leptopus*. Its advantages, however, have already been shown for other plants. Among several investigated extraction techniques on Annona muricata, Tambun et al. reported that the MAE approach gave the best yield of 33.98% compared to maceration and Soxhlet extraction techniques, which proved that MAE is a technique worth investigating.

PHYSICOCHEMICAL ANALYSIS

Phytochemical screening of the crude ethanolic extract of *Antigon Leptopus* Hook & Arn. was carried out for the presence of phytocompounds including terpenoids or steroids, alkaloids, flavonoids, coumarins, saponins, tannins, and anthraquinone using the method described above. The results indicate that the extract contains low amounts of coumarins, anthraquinones, flavonoids, unsaturated sterol, and/or triterpene compounds, a moderate amount of tannins, and a high amount of alkaloids and saponins.

Test for the presence of alkaloids.
Milliliter of extracts and three milliliters of 1% HCl were combined, and the mixture was heated for twenty minutes.

Mayer's examination
One milliliter of Mayer's reagent has been gradually added to one milliliter of plant extract in a test tube. When a cream-colored or greenish precipitate developed, alkaloids were present.

Dragendroff test
Drop by drop, one milliliter of Dragendroff's reagent was added to one milliliter of plant extract in a test tube. When a reddish-brown precipitate formed, alkaloids were detected.

Wagner's examination
In a test tube, 1 milliliter of Wagner's reagent was added drop by drop to 1 milliliter of plant extract. When a reddish-brown precipitate started to form, alkaloids were present.

Test For The Presence Of Saponins
The mixture was quickly shaken after 3 milliliters of plant extract and 3 milliliters of distilled water were added. The formation of a steady, long-lasting foam was regarded as a saponin test result.

Test for the presence of flavonoids
Two milliliters of the plant extract were boiled and dissolved in two milliliters of methanol.

After inserting a magnesium metal chip into the liquid, a few drops of strong HCl were added.

The presence of a reddish-orange coloring was used to identify the flavonoids**.**

Test For the Precence of Tannins
A test tube with around 2 ml of plant extract diluted in 5 ml of distilled water was filled with a few drops of a 1% lead acetate solution. A red precipitate

Test For the Presence of Phenols
Two milliliters of a 5% FeCl3 solution were used to dissolve one milliliter of crude extract. Tannins and phenols were distinguished by their blue-green or black hue. Check for terpenoids. About two milliliters of chloroform and three milliliters of H2O2 were combined with five milliliters of plant extracts. The terpene test result was viewed as having a reddish-brown color.

TOXICITY AND SAFETY

The cytotoxic examination of *A. leptopus* reveals that many in vitro and in vivo studies showed that *A. leptopus* had no significant mutagenic, toxic, or detrimental effects on their model systems. Mamidipalli et al. took albino mice and Wistar rats as models for an acute toxicity study on A. leptopus roots. Several doses were administered to the animals over a 24-hour period, culminating in the highest dose level of 2000 mg/kg body weight. The animals did not exhibit any symptomatology related to toxicity or signs of mortality. Poosa and Vanapatla conducted a similar toxicity study on *A. leptopus* leaves using the test guideline 423 by the Organization for Economic and Cultural Development. The results indicated that *A. leptopus* did not cause any adverse effects or mortality in the rats at up to 2000 mg/kg dosage, which is in agreement with Mamidipalli's results. Prajna et al[22]. conducted a study on cytotoxicity of a hexane fraction of the methanolic extract of *A. leptopus* flowers in vitro on a CHOK 1 cell line (Chinese hamster ovary cell line) and an A-549 cell line (human lung adenocarcinoma epithelial cell line) and reported that there was no cytotoxic effect, thus exhibiting the safety of flowers. All parts of the plant are edible and find their place in Thai cuisine [23]. Overall, the studies have proved that *A. leptopus* does not possess any acute toxic effect

EXTRACTION AND ISOLATION

To prepare *Antigonon leptopus* tea, 5g of dried aerial parts was soaked in 50mL hot water for 6 hours. The resulting tea was evaporated under reduced pressure to give a dark brown residue of 606 mg. This residue inhibited both LPO and COX enzymes at 100 µg/mL. The bioactive extract was analyzed by TLC using CHCl3 and MeOH (4 : 1) as the mobile phase along with the methanolic extract prepared earlier in our laboratory. The TLC patterns of tea residue and methanolic extract were identical except for a new band under UV (R f. 0.63). In order to purify this UV-active compound, an aliquot of the water extract (200 mg) was purified by preparative TLC with CHCl3 and MeOH (4 : 1) as the developing solvents afforded compound 1 (0.7 mg). The structure of this compound was confirmed by 1H and 13C NMR spectral methods.

Methylation of Hydroxy Benzaldehydes
Methylation of hydroxy benzaldehydes followed known procedures. To a round-bottom flask, the monohydroxy benzaldehyde (0.5 mmol) was dissolved in 5 mL of acetone. To this solution, add 1 mmol of anhydrous potassium carbonate, seal with septa, and stir for 10 minutes. Dimethyl sulfate (1 mmol) was injected into the reaction mixture and stirred at room temperature for 6 hours. The reaction was monitored using TLC every 30 minutes. The reaction mixture was evaporated under vacuum. The residue was dissolved in 25 mL of RO water, transferred to a separating funnel, and extracted with 25 mL of ethyl acetate. The ethyl acetate layer was treated with a saturated sodium bicarbonate solution

and evaporated under vacuum. The product was purified using preparative TLC with chloroform: methanol (80:20) as the mobile phase. The procedure for methylating diand trihydroxy benzaldehydes was the same, but using 2 and 3 mmols of potassium carbonate and dimethyl sulfate accordingly. The reaction periods for dihydroxy and trihydroxy benzaldehydes were 12 and 18 hours, respectively. The yields for mono-, di-, and trimethoxy benzaldehydes were 90%, 86%, and 75%, respectively. The chemicals were analyzed using NMR spectral techniques. The NMR spectra of hydroxy benzaldehydes purchased from Sigma-Aldrich are not included in this publication, though they confirm that the compounds were pure.

Plant material

Antigonon leptopus leaves were gathered in several sites around the campus of the University of the Philippines-Diliman from June 2010 to June 2011. Representative plant samples were submitted to the Institute of Biology Herbarium for proper identification and authentication. Collected samples matched the already existing voucher specimen 1809.

Crude extraction

Mature leaves of *A. leptopus* were selected and washed with running tap water. Samples were air-dried atop acid-free paper for 3 days. The dried samples were weighed and homogenized prior to exhaustive extraction with distilled technical grade MeOH. The extracts were filtered and concentrated in vacuo yielding the crude methanolic extract. The methanolic extract was divided into hexane and distilled deionized water (6:1). Solvent partitioning was repeated until a nearly colorless hexane layer was formed and concentrated in vacuo. Then, the aqueous layer was extracted with EtOAc (6:1) several times until an almost colorless organic layer was obtained. The aqueous extract was freeze dried.

Column chromatography for bioactive fractions

These active compounds were isolated by the enzyme-guided isolation and purification strategy as outlined below: The dry weight of the active fraction was recorded, followed by addition of a little amount of MeOH, well mixed until homogeneous, before loading onto the prepared column. 2g aqueous extracts were used every run.50 g of Sephadex LH-20 was swollen in analytical grade MeOH for 24 hrs. prior to loading (2g). A glass column of diameter 3 cm was packed to 60 cm. Isocratic elution with MeOH was conducted. Eluates were collected at a rate of 5 mL per 20 mL scintillation vial.UV-visible profiles of all the eluates were measured from 200 to 700 nm using MeOH as a solvent. Fractions having similar profiles and maximum absorption were pooled together. The isolates were further purified by RP-HPLC with gradient elution of H2O and ACN. Fractions of similar retention durations were collected. Peaks at 5, 20, 34, and 43 minutes were recorded and pooled. Optimized settings are as follows: λAbs = 254 nm; flow rate: 2 mL/min; gradient: 0.01 min = 5% ACN, 5.01 min = 5% ACN, 11.01 min = 11% ACN, 60.01 min = 50% ACN, 75.01 min = 100% ACN, 80.01 min = 5% ACN, and 85.01 min = 5% can.

Characterization

The uncorrected melting point was determined. For FTIR analysis, materials were prepared by the spread-plate technique onto KBr plates. The UV-visible spectra and maximum absorption of all fractions were obtained by dissolving in MeOH and measuring absorbance from 200 to 700 nm. Mass spectra were acquired with a MALDI-CID-TOF/TOF system with a laser intensity of 4100-4300 and 2,5-dihydroxybenzoic acid as the matrix.1H and 13C-NMR, distortion less enhancement by polarization transfer (DEPT), hetero nuclear multiple quantum correlation (HMQC), heteronuclear multiple-bond correlation spectroscopy (HMBC), double quantum filtered-correlated spectroscopy (DFQ-COSY), total correlation spectroscopy (TOCSY), and nuclear over hauser effect spectroscopy (NOESY) were obtained in a 600 MHz

Micropropagation protocol for *Antigonon leptopus* an important ornamental and medicinal plant

Plant materials

Stem nodal explants (2–3 cm) of *Antigonon leptopus* were taken from the unique climbing tree grown at Zohrya Botanical Garden, Zamalek, Cairo, Egypt as explant source for this work.

Culture's establishment stage

Nodal explants from young branches of *Antigonon leptopus* were rinsed in soapy water with septol soap and shaken for 20 minutes before washing with running tap water for an hour. Under aseptic conditions in a laminar air-flow chamber, the explants were then submerged in the following solutions: For 5 minutes, use 20% (v/v) of commercial Clorox (NaOCl, 5.25% free chlorine), 0.1 (w/v) of mercuric chloride (MC), or 10% Clorox for 3 minutes, followed by 0.1 MC for 2 minutes with a few drops of Tween-20. Each treatment comprised of seven jars, each with five nodal explants. Decontamination, survival and shoot formation percentages were recorded after 4 weeks of incubation for this stage.

Culture media and incubation conditions

MS culture media was solidified with 0.7% agar and autoclaved at 1.2 kg/cm2 for 15 minutes. The pH of

the culture media was adjusted to 5.8 by adding 0.1 N KOH or 0.1 NHCL. Culturing was done in 200ml glass jars with 25ml of the media. Cultures were cultured for 4 weeks in a controlled growth chamber. The incubation temperature was 24 ± 2 °C, controlled by a "Power" air conditioner. The photoperiod was set to 16 hours of light and 8 hours of darkness, controlled automatically. Cool fluorescent bulbs (120 cm long) provided an illumination level of 3000 lux.

Shootlet Multiplication Stage

The experiment aimed to investigate the impact of varying concentrations of Benzyl adenine (BA) or 6-(γ, γ- Dimethylallyamino) purine (2iP) (0.5 and 1.0 mg/L) alone or in combination with 0.2 mg/L Indol Butyric Acid (IBA) on shootlet multiplication across three subcultures. The experiment involved 8 treatments, with each combination repeated 7 times (one jar/replicate) in a completely randomized manner. Explants were re-cultured three times at 30-day intervals. Data from each subculture of this stage (shoot formation %, number of shootlets/explant, shootlet length (cm), and number of leaves generated per shootlet) were collected.

Rooting stage

Trials were done to examine the effects of MS-medium (¼, ½, ¾, and full strength) free hormones and MS full strength combined with 1.0 mg/L IBA on shootlet rooting behaviors. After multiplication, uniform shootlets were isolated and cultivated in 7 replicates for 6 weeks. Following that, rooting ability, number of initiated roots, and length of the produced roots were measured.

Ex vitro acclimation step

An experiment was carried out to investigate the impact of six soil combinations on the features of the acclimatization stage. The mixes included sand, clay, peat moss+sand, peat moss +clay, and sand +clay (1:1 v/v ratio). Plantlets (8-10 cm) from in vitro rooting stage were rinsed from agar and put into plastic pots (0.2 liter) containing soil mixtures after saturation with 0.2% Topsin-M70 fungicide in 7 duplicates, each containing three plantlets. The culture pots were coated with white transparent polyethylene bags and kept in a fiber-glass housing. After two weeks of culturing, one pore was completed per plastic bag, and the bags were gradually removed over the next two weeks. The acclimatized plantlets were water irrigated twice a week for four weeks before transplanting out-door. At the end of this experiment, survival percentage, plantlet height and root length were recorded.

Randomly amplified polymorphic DNA (RAPD)

Plant tissues were ground to a fine powder with liquid nitrogen before bulked DNA extraction was performed with the DNeasy plant Mini Kit (QIAGEN). Polymerase Chain Reaction (PCR) amplification was done using five random 10-mer arbitrary primers (synthesized by Operon Biotechnologies, Inc., Germany) with the following sequences: OP-A02 (5′ TGCCGAGCTG 3′), OP-B07 (5′ GGTGACGCAG 3′), OP-B09 (5′ TGGGGGACTC 3′), OP-B11 (5′ GTAGACCCGT 3′), and OP-C19 (5′ GTTGCCAGCC 3′).

The following reagents were used in a 25 µl reaction "volume": The following was used: 2.5 µl of dNTPs (2.5 mM), 2.5 µl of MgCl2 (2.5 mM), 2.5 µl of 10 x buffer, 3.0 µl of primer (10 pmol), 3.0 µl of template DNA (25 ng/µl), 1 µl of Taq polymerase (1U/µl), and 10.5 µl of sterile "dd" H2O. The DNA amplification was performed in an automated thermal cycle programmed for one cycle at 94°C for 4 minutes, followed by 45 cycles of 1 minute at 94°C, 1 minute at 36°C, and 2 minutes at 72°C. The reaction was kept at 72 °C for 10 minutes. The amplified products were size-fractionated using a 50-bp ladder marker and electrophoresed in a 1.5% agarose gel. The relationships among mother plants and micropropgated plants were done using SPSS windows (Version 10) program. DICE computer package was used to calculate the pairwise difference matrix among lines.

Experimental design and data statistical analysis

The experimental design for shot "establishment" rooting and acclimation stages was designed as one factor in a completely randomized design, whereas the experiments on shootlet multiplication stage were set up as two factorials in a completely randomized manner. Data from the three studies were averaged and analyzed statistically using analysis of variance. Means comparisons at 5% levels were done using Dancan's multiple range test by Steel and Torrie.

CYCLOOXYGENASE ENZYME INHIBITORY ASSAY

The Cyclooxygenase enzyme inhibitory assay was performed using the described procedure. The COX-1 enzyme was produced using ram seminal vesicles bought from Oxford Biomedical Research, Inc. (Oxford, MI). COX-2 enzyme was produced by diluting insect cell lysate with buffer (pH 7) to achieve a final concentration of 1.5 mg protein/mL. The activities of phenolic compounds were evaluated by measuring the initial rate of O2 absorption at 37°C using a micro-oxygen chamber and electrode (Instech Laboratories, Plymouth Meetings, PA) connected to a YSI model 5300 biological oxygen monitor (Yellow Springs Instrument, Inc., Yellow Springs, OH). The assay mixtures included buffer (0.6 mL, 0.1 M, pH 7), phenol (1 mM), hemoglobin (85 µg), and DMSO/test

samples (10 µL). To activate the reaction, 20 µL of COX-1 or COX-2 enzymes were introduced to the chamber and incubated for 3 minutes.

Arachidonic acid (10 µL of a 1 mg/mL solution) was then added. Each sample was analyzed in duplicate, and the standard deviation was computed for n = 2. The data were collected using QuickLog for Windows, a data gathering and control software (Strawberry Tree, Inc., Sunnyvale, California). Compounds 2–24 were evaluated at a concentration of 100 µg/mL. The percentage inhibition was calculated relative to the DMSO control. Compound 1 was evaluated at 25 µg/mL and serially diluted to create a dose-response curve. Positive controls included non-steroidal anti-inflammatory medications such as aspirin (60 µM), Celebrex (26 nM), and Vioxx (32 nM). Aspirin was obtained from Sigma-Aldrich Co. (St. Louis, MO), while Celebrex and Vioxx were physician samples generously donated by Dr. Subash Gupta of Sparrow Pain Centre, Michigan, USA. We solely utilized Vioxx as a positive control in our in vitro assays. We continue to utilize Vioxx as a positive control in in vitro tests since it is a particular inhibitor of the COX-2 enzyme among nonsteroidal anti-inflammatory drugs (NSAIDs) that are manufactured and marketed. The inclusion of Vioxx as a positive control in our in vitro assay is solely for comparison and not as a treatment. It is only used to investigate the mode of action of test substances.

LIQUID PEROXIDE INHIBITORY ASSAY

The in vitro lipid peroxidation assay was performed as described, by using large unilamellar vesicles (LUVs) and fluorescence spectroscopy. The phospholipid, 1-stearoyl-2-linoleoyl-sn-glycero-3-phosphatidylcholine (SLPC), in CHCl3, and the fluorescent probe, 3-[p-(6-phenyl)-1,3,5-hexatrienyl] phenylpropionic acid (DPH-PA), in DMF (mg/mL), were homogenized and dried under reduced pressure. The LUVs were prepared by suspending the lipid-probe mixture (0.15 M NaCl, 0.1 mM EDTA, and 0.01 M MOPS buffer over Chelex resin), followed by ten freeze-thaw cycles in a dry ice-EtOH bath and 29 times extrusion over a 100 nm pore-size membrane (Avestin Inc., Ottawa, Canada).

- The final assay volume was 2 mL, which contained 100 µL of HEPES buffer, 200 µL of 1M NaCl, 1.64 mL of N2-purged water, 20 µL of test sample, and 20 µL of liposome suspension. The induction of lipid peroxidation was achieved with 20 µL of FeCl2 · 4H2O (0.5 mM). The fluorescence was measured using a Turner Model 450 Digital Fluorometer at 0 min, 1 min, 3 min, and every 3 min for 21 min. The decrease in relative fluorescence intensity over time was used to calculate the rate of peroxidation. The % inhibition was calculated relative to the DMSO control. The extracts were tested at 100 µg/mL. Compound 1 exhibited more than 50% activity, therefore pure compounds were assayed at 6.25 µg/mL. Commercial antioxidants BHA (butylated hydroxyanisol), BHT (butylated hydroxytoluene) and TBHQ (t-butyl hydroquinone) were evaluated at 1 µg/mL

CONCLUSION

Although *A. leptopus* is considered an invasive vine in Guam, it also has been used in traditional medicine to cure colds, stomach flus, skin irritations, and other ailments. Its various ethnomedicinal uses have prompted the study of phytochemical constituents and bioactivities of this plant. *A. leptopus* contains bioactive compounds, including polyphenols, flavonoids, terpenoids, alkaloids, glycosides, and tannins showing pharmacological activities such as antioxidant, antibacterial, antidiabetic, antinflammatory, and anticancer activities with no immediate cytotoxicity. Conventional approaches remain the most common method of bioactive chemical extraction from *A. leptopus*; however, specific studies on green technology applications, such as UAE, MAE, and S-CO2 extraction techniques, are lacking. Biological studies and standardized extraction methods need to be conducted to identify and characterize the specific components responsible for the pharmacological properties of *A. leptopus*. Despite its ecological importance, the Marianas currently has only limited and outdated information on this species. *A. leptopus* had been a potential source of bioactive compounds for the nutraceutical industry. Future research must be focused on the identification and isolation of its phytochemicals through green approaches in order to define their commercial potential for applications in the region.

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